Title:

Evidence for mid-Holocene rice domestication in the Americas

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The development of agriculture is one of humankind's most pivotal achievements and questions about plant domestication and the origins of agriculture have engaged scholars for well over a century, with implications for understanding its legacy on global subsistence strategies, plant distribution, population health, and the global methane budget . Rice is one of the most important crops to be domesticated globally, with both Asia (*Oryza sativa* L.) and Africa (*Oryza glaberrima* Steud.) discussed as primary centres of domestication. However, until now the pre-Columbian domestication of rice in the Americas has not been documented. Here we document the domestication of *Oryza* sp. wild rice by the mid-Holocene residents of the Monte Castelo shell mound starting at *ca.* 4000 cal yr BP, evidenced by increasingly larger rice husk phytoliths. Our data provide evidence for the domestication of wild rice in a region of the Amazon that was also the likely cradle of domestication of other major crops such as cassava (*Manihot esculenta*), peanut (*Arachis hypogaea*) and chilli pepper (*Capsicum* sp.). The results underlines the role of wetlands as prime habitats for plant domestication worldwide.

More than half of the world's population depend on rice for more than 20% of their daily calories [1]. Modern global consumption is dominated by varieties of the domesticated Asian (*Oryza sativa* L.) and African (*O. glaberrima* Steud.) species [2], which were domesticated in the early Holocene in the Yangtze River, China[3], and ca. 2000 cal yr BP in West Africa [4]. In North America, *Zizania* wild rice was so important to the subsistence economy of several Upper Great Lakes Native American tribes that some early-twenty century ethnologists designated this region as a distinct 'wild rice culture area'[5]. Wild rice was already a seasonal staple of indigenous subsistence in South America long before the introduction of Old World species in the 18th century[6]. Growing in seasonally flooded areas that compose up to 10% (1.4M km^2) of lowland South America (Fig. 1), wild rice is a particularly important resource during the rainy season when flooding causes other resources to be dispersed and scarce[7]. Early 16th-19th century historical and ethnographic accounts report extensively on the consumption of wild rice species by indigenous groups in this region. Similar to the traditional North American canoe-and-flail harvesting method, native South American people were reported to harvest wild rice by beating the grains of mature inflorescences into their canoes with wooden poles[8-11]. South American accounts hint towards the importance and culinary practices involving wild rice. For example, De Azara[12] mentions the consumption of an unknown type of rice in southern Paraguay that "… feed a nation of

approximately seventy warriors". Cardim[13] mentions that wild rice was mixed with maize to make bread, and Acosta[14] describes its consumption in the form of a fermented brew, similar to wine. Locally known as "arroz-de-pato" (duck rice) or "arroz-do-brejo" (swamp rice) today, wild rice is stilly consumed as a valuable source of carbohydrates when other food resources are scarce by riverine communities across the Amazon. It is still gathered and consumed in various modern localities close to the study site along the Guapore River such as Costa Marquez and Santo Antônio, where the communities used to manage wild rice stands until the first half of the twentieth century. This kind of landscape management can still be observed in other parts of the Amazon, such as in wild rice fields of the municipality of Manaquiri, in the lower Solimoes river basin[15] (Supplementary Figure 1b). In the Pantanal, the native Guató communities consume the wild native species *Oryza glumaepatula* and *O. latifolia* by sun drying the seeds, peeling them, and boiling them [16]. However, despite the occasional reference to its potential role in pre-Columbian diets [17,18], the domestication of rice has not yet been investigated in this region. Increasingly larger *Oryza* sp. husk phytoliths recovered from mid-Holocene levels of a shell mound in southwestern Amazonia (Fig. 1) dating to ca. 4000 cal yr BP show the progressive selection of larger wild rice seeds by its pre-Columbian residents, whom were already engaged in the cultivation of maize (*Zea mays*) and squash (*Cucurbita* sp.) [19].

Taxonomy and domestication phytoliths of the *Oryza*.

Oryzoideae (syn. Ehrhartoideae) is a subfamily of the true grass family Poaceae that includes around 120 species in 20 genera. The Oryzeae tribe within the Oryzoideae subfamily consists of twelve genera and is distributed in tropical and temperate regions worldwide. Five of these twelve genera occur in South America: *Leersia*, *Luziola*, *Rhynchoryza*, *Zizaniopsis*, and *Oryza* [20,21]. The *Oryza* genus comprises 22 known wild species. Four of them are endemic to Latin America with a tropical-subtropical distribution from Cuba 23^0 N to the Paraná River delta 34^0 S, including the diploid (2n = 24, AgpAgp) *O. glumaepatula*, and three tetraploids (2n = 48, CCDD) *O. alta*, *O. grandiglumis* and *O. latifolia*[22] (Fig.1). *Oryza* spp. rice are all aquatic emergent macrophytes that grow along rivers, lakes and wetland margins. *Oryza alta*, *O. grandiglumis*, and *O. latifolia* are perennial species, while *O. glumaepatula* can be annual, biannual or perennial depending on the geographical location [23,24]. *Oryza* spp. have a nutty flavour, and firm consistency. Preliminary studies on *O. glumaepatula* show that it has high levels of total protein, albumin, and glutelin fractions, which compares favourably with *O. sativa* commercial cultivars[25]. Wild rice can

also be stored and can be rather productive. Although, not directly comparable to *Oryza*, the traditional canoe-and-flail harvesting of *Zizania* wild rice in North America yield about 125 kg/ha[26], while modern domesticated shattering resistant cultivars, yields have been reported as high as 1,680 kg/ha in Minnesota and twice that amount in California[27].

The Oryzoideae subfamily produce four distinct phytoliths associated with different parts of the plant. The Oryzeae tribe produce: 1) cuneiform keystone bulliform cell phytoliths exhibiting fish-scale decorations on the fan edges are produced in the leaves (Fig. 2d) and 2) 'scooped'-shaped bilobates in the leaves and stems (Fig. 2e). The *Oryza* genus produce: 3) double-peaked glume cells (Fig. 2a-b, f-i); and 4) deeply serrated phytoliths both derived from the epidermis of the *Oryza* seed glume (husk) (Fig. 2c)[28-30]. The presence of diagnostic *Oryza* phytoliths produced in the different parts of the plant has allowed the detection of crop processing stages[31] and different agricultural techniques[32] in Asia. For example, the distinctive bulliform and bilobate phytoliths from Oryzeae leaves and stalks are representative of the early stages of harvesting and processing, while the *Oryza* husk double-peaked glumes represent later stages of processing, such as pounding, winnowing and storage.

Domestication is a process that causes genetic changes in populations such that the average phenotype diverges from the range found in wild populations[18]. Domestication causes a gradual increase in plant size from wild to domesticate as a result of selective exploitation[33]. As the plant become larger, so do the phytoliths. The increase in phytolith size has been documented in *Zea mays*[34], *Cucurbita*[35] and *Musa bananas*[36], where larger fruits and seeds often yield considerably larger phytoliths. Pearsall [29] and Zhao et al.[30] have demonstrated a clear correlation between increasing phytolith size and domestication in Asian rice based on the analysis of 27 accessions of domestic rice, originated from China, and 79 specimens from the nine wild rice species considered ancestral to rice distributed geographically in South and Southeast Asia. These authors[30] devised a discriminant function to differentiate assemblages of wild from domesticated *Oryza* rice using five different size measurements of the double-peaked glume cells including: 1) Top Width (TW): the distance between the two peaks of the projecting hairs; 2) Maximum Width (MW): the width at the point where the glume projection attaches to the base; 3-4) Height of each hair (H1, H2): length from the tip to the base of the hair, H2 is defined as the smaller measurement; and 5) the Curve Depth (CD): distance from the tip of H1 to the lowest point of the curve (Fig. 3m). Further comparative research by Lu et al.[37] including hundreds of grass species from China

and Gu et al[28] including wild and domesticated rice species from East Asia have confirmed their results.

Archaeological background: the Monte Castelo shell mound. Dating back to ca. 10,000 cal yr BP, a diversity of coastal and freshwater [38] shell mounds represent some of the oldest forms of human occupations across lowland South America, some of which are associated with the earliest ceramics on the continent[39]. Our study site, the Monte Castelo residential shell mound is located in the Upper Madeira basin of SW Amazonia, Rondônia state, Brazil. The region is characterised by a seasonally flooded tropical wetland exhibiting gallery forest along the larger streams, which are dotted with anthropogenic shell mounds [38]. Monte Castelo is a 6.3 m high platform-shaped freshwater shell-mound, exhibiting a 160 m long elliptical base (Fig. 1c) and dating from 9400 cal yr BP[40,41]. The first excavation of Monte Castelo by Miller [42] in 1984, revealed a seven-meter-deep stratigraphy bracketing a long-term occupation from 9130 to 667 cal yr BP (Supplementary Table 1). Miller defined three major and one transitional occupation phases based on stratigraphy, artefact content and sixteen radiocarbon dates including: Cupim phase (700-685 cm; 9130-7701 cal yr BP), Sinimbu phase (670-275cm; 7701-4822 cal yr BP), Sinimbu-Bacabal transitional stratum (275-220 cm; 4862-4388 cal yr BP) and Bacabal phase (220-30 cm; 4388-689 cal yr BP)[42]. Renewed excavations at Monte Castelo in 2014 and 2016 by the Laboratory of Tropical Archaeology of the University of São Paulo expanded the previous excavation by E. Miller reaching a depth of 640 cm. They uncovered ten archaeological strata across the Sinimbu to Bacabal phases dating from 5310 cal. yr BP. to 689 cal yr BP (Fig. 3k; Supplementary Figure 3; Supplementary Note 1)[40]. The stratigraphy shows a sequence of construction events evidenced by unburnt entire *Pomacea* shell layers, occupation floors marked by lenses of crushed shells, primary burials and human-created dark soils. Sample collection for microfossil analysis was carried out in undisturbed sectors of each of the layers and targeted samples were collected from particular features such as burials (Supplementary Note 1; Supplementary Figure 3; Supplementary Table 3).

Results and Discussion

To investigate the use and potential domestication of wild rice by the Monte Castelo residents we analysed both archaeological samples and modern wild rice reference material. A total of 16 archaeological sediment samples, from across all ten levels uncovered during the 2014 Monte Castelo excavations (Fig. 3; Supplementary Table 3), and 19 modern

specimens from the four wild species of rice occurring in South America (Supplementary Table 2), were analysed for phytoliths following standard procedures[34] (Methods, Supplementary Table 2 and 3). Each slide was scanned until the first 20 double-peaked glume cells were encountered. Following Zhao et al.[30], the five metric attributes (Fig. 3m) were measured from 20 *Oryza* double-peaked glume phytoliths from each of the archaeological (16) and modern samples (19) totalling 700 phytoliths.

Phytolith preservation was excellent in all context analysed. All archaeological sediment samples analysed yielded phytoliths of wild rice. Our analysis shows a clear increase in the proportion of rice morphotypes in the total phytolith assemblage from 6.4% on average in the Sinimbu phase occupation (Layers J-H) to 14.4% in the more recent Bacacal phase, suggesting that rice may have played a larger role in diet over time (Fig. 3f).

At Monte Castelo, there is also an increase in the proportion of *Oryza* seed phytoliths from the lower to the upper levels of the mound reflected in the husk:leaf+stem ratio. For example, during the Sinumbú phase (Layers J-I; 280-460cm) *Oryza* sp. seed phytoliths represent on average 3.4% of the total assemblage while Oryzeae leaf and stem phytoliths constitute on average 3%, a 1/1 ratio. During the Bacabal occupation (Layers F-A; 30-210 cm) *Oryza* seed phytoliths constitute on average 12% of the total assemblage while leaf phytoliths constitute on average 3.5%, a ratio of 3.4/1, over three times the relative proportion of seed husks as occur in the Sinimbu occupation (Fig. 3g). The collection and flailing of wild rice in canoes in the Americas should leave leaf and stem bulliform and bilobate phytoliths in the place of harvest while double-peaked and deeply serrated glume phytoliths should be more abundant at residential sites where the grain is brought for consumption. Therefore, the increase in the ratio of husk:leaf+stem Oryzeae phytolith morphotypes suggests that the Monte Castelo residents became more efficient harvesters over time, bringing more grain and fewer leaves to the site.

The analysis of the average size of the attributes measured on the *Oryza* glume phytoliths (Fig. 3 and Supplementary Figure 5) shows a gradual increase in Height (H1, H2) and Width (TW, MW) through time. Mean H1 values increase ca. 8µm (17µm to 25µm) and H2 increases ca. 7µm (15µm to 22µm) from Layers J to A. MW increases 9µm (48-57µm) through the stratigraphy. Mean CD values are larger in the upper occupation layers (A-H) compared to its initial dimensions in Layers I-J (Fig. 3). We used Principal Component Analysis (PCA) of modern reference wild species to determine the variables that best explained phytolith shape differences among specimens, which are the two highly correlated height and width measurements (Supplementary Note 2, Supplementary Figures 6-9).

Following Zhao et al.[30], therefore, we created a simple model of phytolith size to characterise the changes in phytolith morphology through time. Results of a one-way ANOVA show that mean phytolith size varies significantly among layers and pairwise comparison (with Bonferroni corrected p-value) shows phytoliths in the upper archaeological layers (A - D) are significantly larger than those in Layer J and wild reference specimens (Supplementary Table 4). Fig. 4 illustrates mean height and width of all *Oryza* phytolith specimens, showing an increase in phytolith size through time. The data show a significant shift towards bigger phytoliths compared to wild specimens began in Layers D-E (Fig. 3k) around 4000 cal yr BP. Phytolith size in lower archaeological layers were not significantly different from some botanical specimens (*O. latifolia, O. alta*) (Supplementary Table 4). The gradual increase in *Oryza* husk phytolith dimensions since the basal layers of the Monte Castelo shell mound suggest that the Monte Castelo residents may have been manipulation *Oryza* by at least 5000 cal yr BP. Phytolith data also show that subsistence strategies of the Monte Castelo residents were based on a mixture of wild and domesticated resources including cultivars such as maize and squash as well as other plants of economic importance including palm fruits and possibly soursop (*Annona* sp.) (Fig. 2 j-m).

Our results indicate a significant increase in the size of double-peaked glume phytoliths across the Monte Castelo occupation starting around 4000 cal yr BP. Wild rice constituted an important seasonal resource for the Monte Castelo residents, who began to husband wild rice stands at lake or river edges. The phytolith data show that wild rice was modified by human intervention to produce larger grains, exceeding the range of variation found in the lower levels of the Monte Castelo shell mound and the modern populations of wild rice. The possibility that the increase in dimensions of husk phytoliths may be a result of selection for large seeds during collection from wild plant stands is countered by fact that no husk phytoliths with larger dimensions than the domesticated ones have been found on the modern wild rice specimens.

Oryza alta, *O. grandiglumis*, and *O. latifolia* are perennial species, while *O. glumaepatula* can be annual, biannual or perennial depending on the geographical location [23,24]. Although we cannot distinguish specific *Oryza* species using phytoliths, it is likely that the Monte Castelo residents were targeting the annual varieties of *O. glumaepatula* due to their generally larger-scale seed production compared to perennials, as seen with other cereal grains[43]. The specific husbandry practices that led to this process of domestication are unknown; however, native North Americans increased natural *Zizania* wild rice stands by mixing wild rice seeds into clay, rolling it into a ball and dropping the clay ball into the

water[27]. It is not unlikely that the Monte Castelo residents may have seeded the Guapore basin wetland margins with a similar practice. With this technique, larger seeds might have been indirectly selected because they would germinate better from the clay balls, eventually leading to domestication. In addition, like traditional societies in India today, they may have practised burning of enriched rice patches during the dry season to remove competing vegetation after rice grains were embedded safely in the soil. To what extent the selection of non-shattering types contributed to the fact that the Monte Castelo residents became more efficient harvesters, as shown by the increase in husk:leaf+stem ratio, is something we cannot directly detect with phytolith analysis, since phytoliths cannot document the presence/absence of this key domestication syndrome trait.

It is interesting to note that the apparent major role of rice in the diet of the Monte Castelo residents, as well as the beginning of its domestication, coincides with a rapid increase in precipitation in the Amazon. As summarised by Iriarte et al.[44], the palaeoclimate records from southern Amazonia and adjacent regions influenced by the South American Low Level Jet show a consistent long-term trend of increasing precipitation starting during the mid-Holocene (~6k cal. yr BP), showing a rapid rise up to 4k cal. yr BP, and then continued to increase slightly towards the present. This higher precipitation would likely have expanded the spatial extent of wetlands across the basin and possibly made the flooding season longer. Since wild rice is a particularly important resource during the rainy season in wetlands and floodplains when flooding causes other resources to be disperse and scarce, the increase precipitation would have likely made wild rice a critical seasonal resource, which may have, in turn, led populations to focus on its manipulation, which ultimately led to its domestication. Further work is needed on this hypothesis.

The presence of phytoliths from known cultigens, such as the wavy-top rondels of maize and scalloped spheres from squash, in the strata analysed shows that both crops were commonly grown in the region from at least 5300 B.P. onwards (Fig. 3, Supplementary Figure 4). This in turn, indicates that the Monte Castelo shell mound residents began to systematically select larger rice seeds when they were already engaged in the cultivation of maize and squash. While in other regions of the Americas, wild grasses such as *Setaria*[45] or marsh-eleder[46] decrease in importance or are replaced by maize, the opposite trend is apparent in the Monte Castelo record. Wild rice was domesticated and increased in importance a considerable time after Monte Castelo residents had become engaged in farming practices.

The arrival of Europeans to the American continent in AD 1492, with the consequent population decimation and impact on cultural practices, caused the domesticated traits to gradually disappear. The loss of domesticated varieties is a phenomena that has also occurred for other indigenously domesticated species in both South[18] and North America[46]. A case in point similar to *Oryza* is the 'extinct cultigen' marsh-elder (*Iva annua*), a member of the Asteraceae family greatly appreciated for its achene oil content, which was originally domesticated in southeastern North America and then abandoned with the introduction of maize[46]. As in our case study, the achenes of marsh elder from the earlier archaeological sequences are not much larger than the modern ones, but the achenes from the more recent archaeological contexts are much larger than any existing races of *Iva annua* today. In the case of rice, some varieties are in the process of de-domestication today; modern studies of Californian weedy rice show how reversions to non-domestic or wild-traits (such as seed shattering, presence of awns) can occur following abandonment[47]. In our case study, it is likely that the wind-pollinated wild rice progressively hybridised with the domesticated one, with the consequent return to the wild characteristics seen today.

Our study highlights the importance of wetlands for the adoption and intensification of agriculture [48,49]. The results contribute to a broader understanding of how wetlands and the seasonal tropical forests of the Amazon may have been critical for early human settlement and the origins of food production in the Americas. This domestication process took place in a region that was likely the cradle of domestication for cassava, peanuts and chilli peppers pointing to the importance of this region of South America[19].

Our research has implications for sustainable Amazonian futures. Modern intensive breeding for high yield and pest resistance has narrowed the genetic diversity of cultivated rice leaving crops more susceptible to disease and less adaptable to the effects of climate change. Understanding the process of rice manipulation by ancient Native Americans and the role of South American native varieties could help provide more resistant high-yielding varieties, and provide further knowledge for plant breeders interested in the introgression of genes from wild *Oryza* species into modern rice varieties[22].

Methods

Phytolith analysis. Phytoliths were identified and counted under a Zeiss Axioscope 40 light microscope at 500X magnification. Phytolith identifications were made using published material for the Neotropics and the Oryzoideae family[29,30,34] and by direct comparison with the phytolith reference collection of the Archaeobotany and Palaeoecology Laboratory in the

Department of Archaeology of the University of Exeter. A minimum of 200 phytoliths were counted per slide. Following Zhao et al.[30], the five metric attributes (Fig. 3m) were measured from 20 *Oryza* double-peaked glume phytoliths from each of the archaeological (16) and modern samples (19) totalling 700 phytoliths.

Figure 1. **a**. Distribution of *Oryza* species, wetlands in South America, and important early Holocene shell mound sites in South America . Species occurrences from the Global Biodiversity Information Facility[50]. Wetland areas from the Global Lakes and Wetlands Database, World Wildlife Fund (https://www.worldwildlife.org). **b.** Map showing the location of the Monte Castelo. **c.** The Monte Castelo locality, topographical map, and location of the 2014 trench excavation.

Figure 2. Microphotographs of phytolith morphotypes recovered at the Monte Castelo shell mound and modern reference wild rice species analysed. **a-e**. *Oryza* sp. phytolith morphotypes recovered in the Monte Castelo shell mound: **a.** double-peaked glume (Layer A); **b.** double-peaked glume (Layer J); **c.** deeply serrated body (Layer C); **d.** cuneiform keystone bulliform (Layer D 130-140cm); **e.** scooped bilobate (Layer E). **f-i.** Double-peaked glume phytoliths from modern wild-rice species native to the study area: **f.** *O.alta* (PRI-1); **G.** *O.latifolia* (Arg-5); **h.** *O.grandiglumis* (SO-23); **i.** *O.glumaepatula* (SO-17). **J-N.** Crops and other native edible plants recovered in the Monte Castelo shell mound: **J.** scalloped sphere from the rind of squash (*Cucurbita* sp.)(Layer F); **k.** wavy-top rondel from the cob of maize (*Zea mays*)(Layer C); **l.** large globular echinate from Arecaceae (Layer J); **m.** conical to hat-shaped phytolith from Arecaceae (Layer H); **n.** spherical facetate from Annonaceae (Layer C); Scale bar= 20 µm.

Figure 3. Sketch stratigraphic diagram of the 2014 Monte Castelo shell mound excavation layers showing: **a-e.** Mean and 95% confidence intervals of the metric attributes of *Oryza* sp. double-peaked glume phytoliths (N=700): **a.** TW, Top Width, **b.** MW Maximum Width, **c.** CD, Curvature Depth, **d.** H1, Height 1 and **e.** H2, Height 2; **f.** Percentage of rice phytoliths to total phytolith assemblage; **g.** *Oryza* husk:leaf+stem ratio; **h.** Presence of *Cucurbita* scalloped spheres; *i.* Presence of *Zea mays* wavy top rondels; **j.** Monte Castelo stratigraphy; **k.** Sketch drawings of double-peaked glume phytoliths using the average of the five metric attributes for each archaeological layer; **l.** Monte Castelo cultural chronology; **m.** Metric attributes of *Oryza* double-peaked glume phytoliths. Box and whisker plots for all metrics are shown in Supplementary Figure 5.

Figure 4. Mean height (H1+H2/2) and width (MW+TW/2) of all Oryza phytolith specimens (N=700), shown with 95% confidence intervals, demonstrating that archaeological specimens are larger compared to botanical specimens, and an increase in phytolith size through time.

Data availability. The dataset analysed is available from corresponding author upon request.

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Author contributions

LH, JI and EGN designed research; EGN, FP, MS and CAZ performed archaeological excavations at Monte Castelo; LH undertook phytolith analysis; BSW carried out statistical analyses; EV provided *Oryza* reference collection samples for analysis; JI and LH led the writing of the paper with inputs from all other authors.

Additional information

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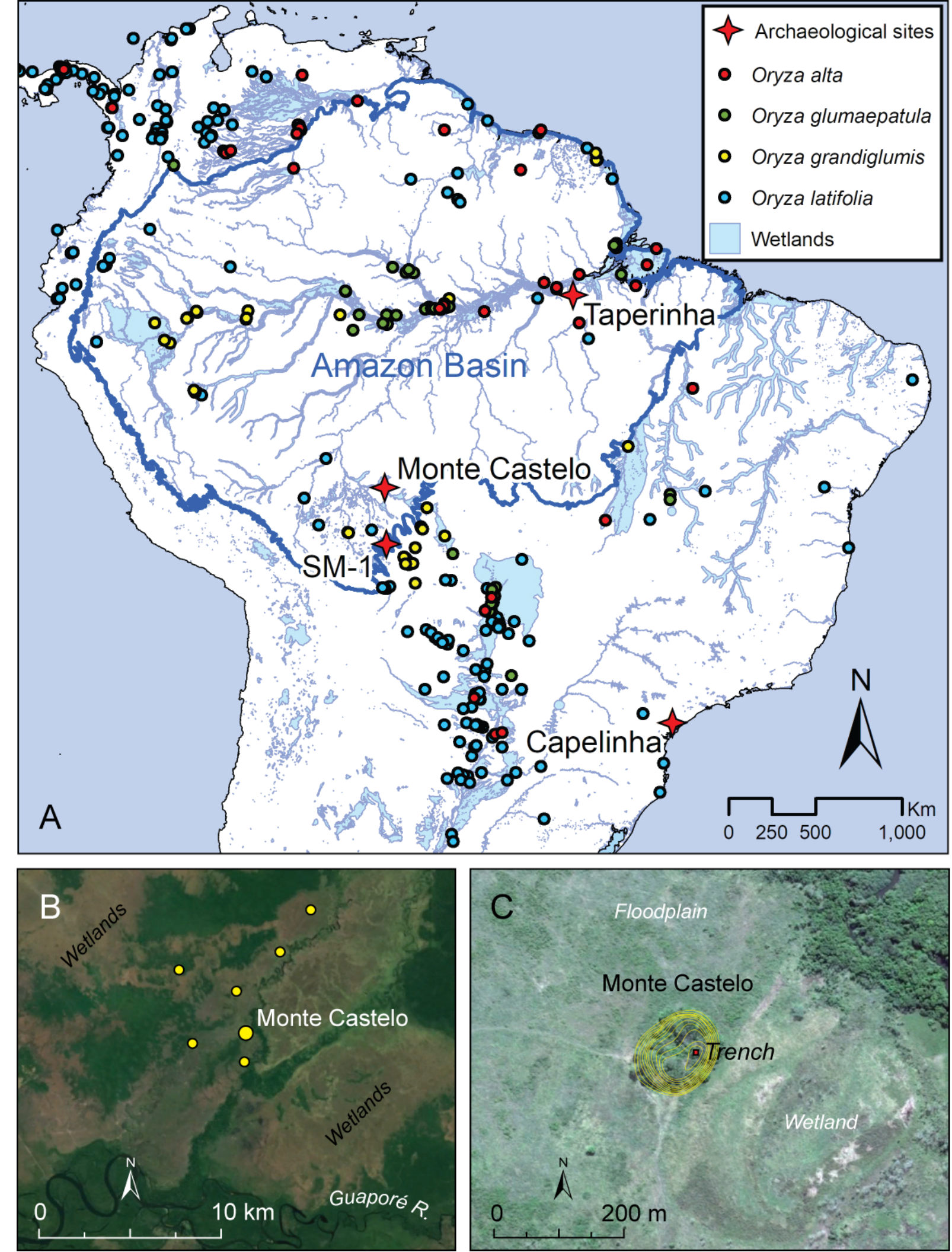
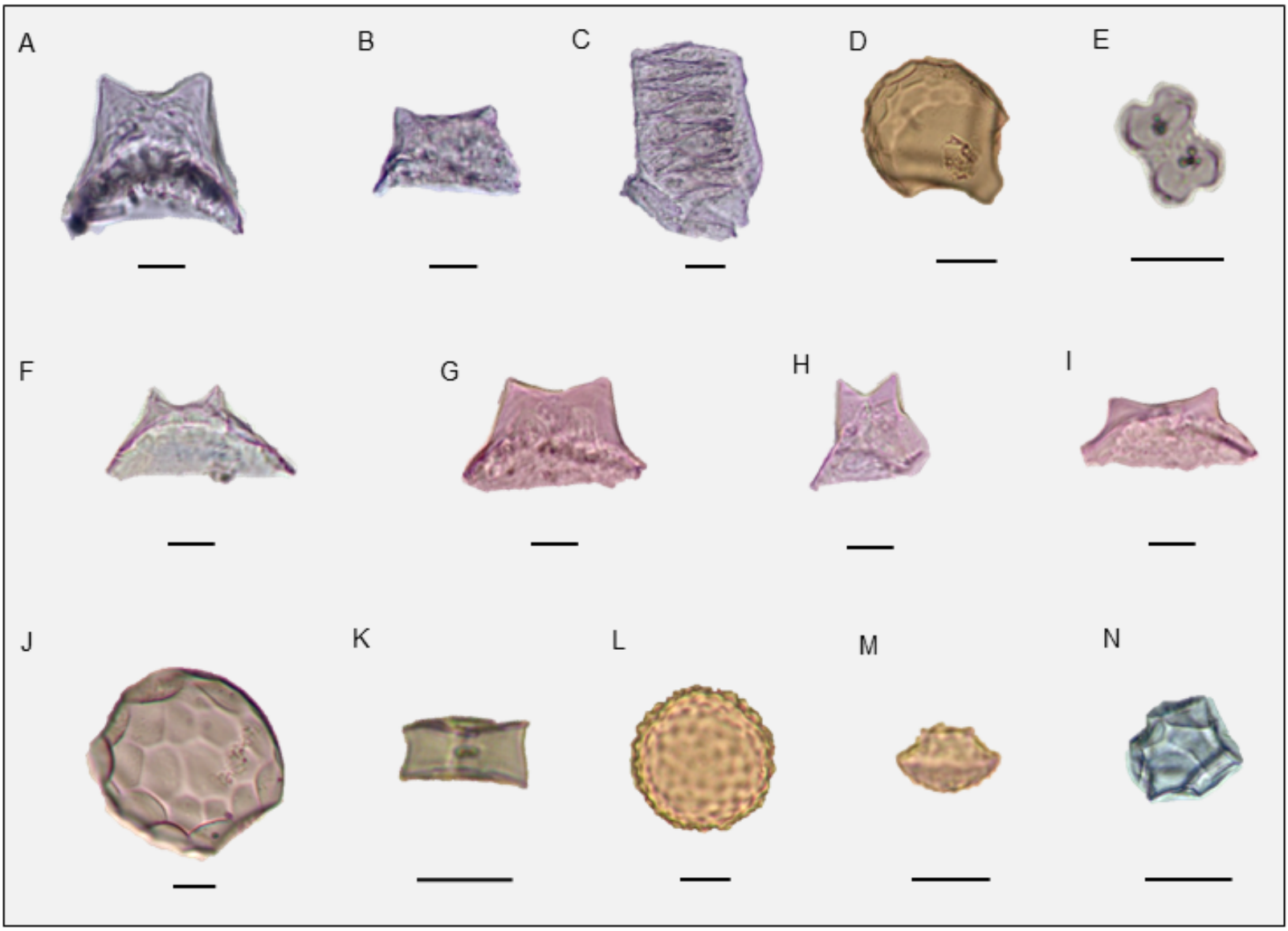
Competing interests

The authors declare no competing financial interests.


Archaeological sites
Oryza alta
Oryza glumaepatula
Oryza grandiglumis
Oryza latifolia
Wetlands
Taperinha
Amazon Basin
Monte Castelo
SM-1
Capelinha
N
Km
0 250 500 1,000
A
B
Wetlands
Monte Castelo
Wetlands
N
0 10 km
Guaporé R.
C
Floodplain
Monte Castelo
Trench
Wetland
N
0 200 m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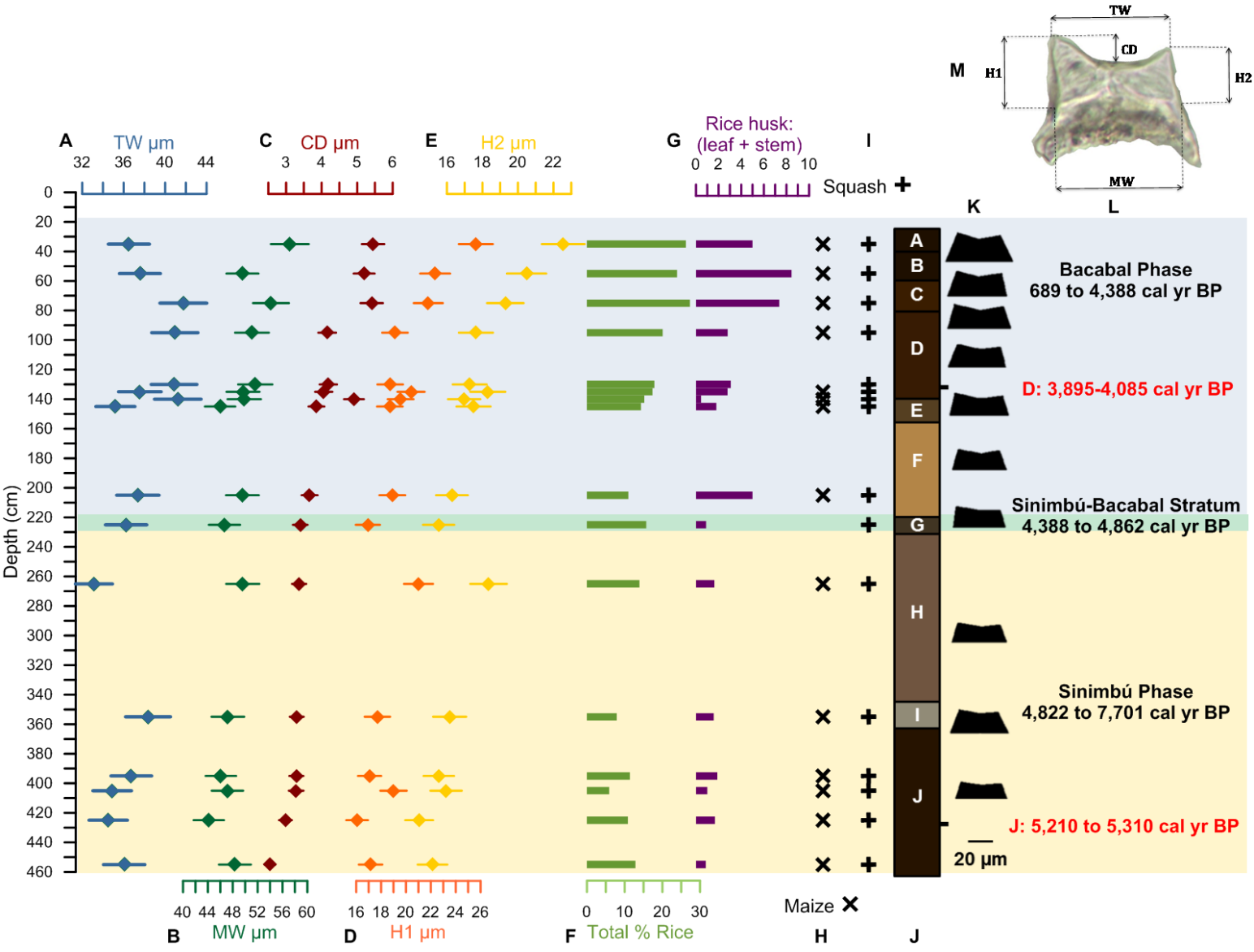
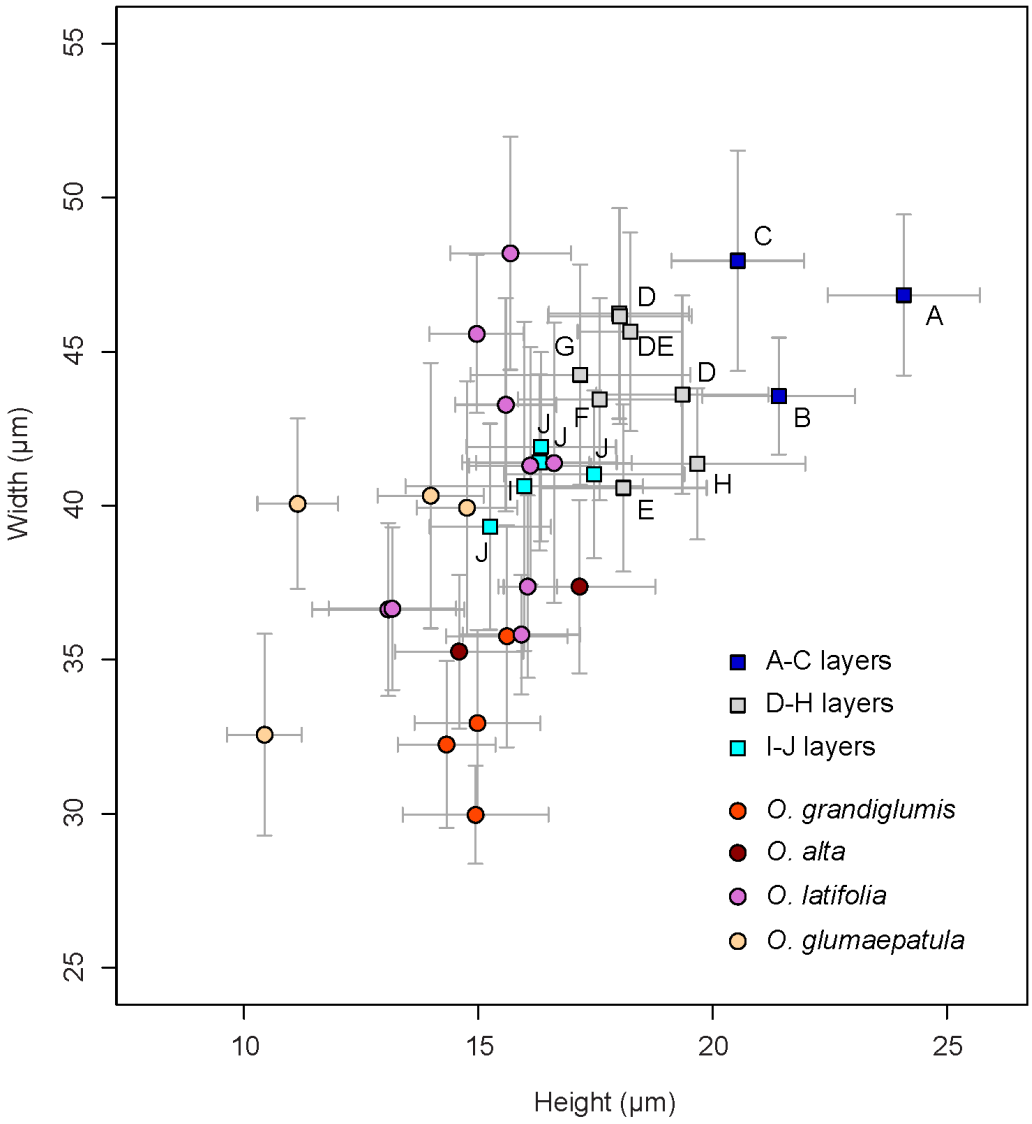

A
TW µm
32 36 40 44
C
CD µm
3 4 5 6
E
H2 µm
16 18 20 22
G
Rice husk:
(leaf + stem)
0 2 4 6 8 10
I
Squash
M
TW
CD
H1
H2
MW
K
L
Depth (cm)
0 20 40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460
A
B
C
D
E
F
G
H
I
J
Bacabal Phase
689 to 4,388 cal yr BP
D: 3,895-4,085 cal yr BP
Sinimbú-Bacabal Stratum
4,388 to 4,862 cal yr BP
Sinimbú Phase
4,822 to 7,701 cal yr BP
J: 5,210 to 5,310 cal yr BP
20 µm
40 44 48 52 56 60
B
MW µm
16 18 20 22 24 26
D
H1 µm
0 10 20 30
F
Total % Rice
Maize
H
J


Width (µm)
Height (µm)
25
30
35
40
45
50
55
10
15
20
25
A
B
C
D
DE
D
E
F
G
H
I
J
J
J
J
A-C layers
D-H layers
I-J layers
O. grandiglumis
O. alta
O. latifolia
O. glumaepatula